Murine Epidermal Vγ5/Vδ1–T-Cell Receptor$^+$ T Cells Respond to B-Cell Lines and Lipopolysaccharides

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The Vγ5/Vδ1$^+$–T-cell receptor (TCR)–bearing T-cell clone, 2CBET-3, was generated from C57BL/6 mice. Upon stimulation, 2CBET-3 cells produce interleukin (IL)-3, granulocyte–macrophage colony-stimulating factor, and tumor necrosis factor-α, but not IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, macrophage colony-stimulating factor, or interferon-γ. These cells were evaluated for their ability to be stimulated by a variety of murine cell lines, including fibroblasts, trophoblasts, melanoma cells, embryonic carcinomas, B-cell lymphomas, mastocytoma cells, and keratinocytes. The human B-lymphoma cell line, Daudi, also was included in these studies. We found that 2CBET-3 cells produced cytokines up to several hundredfold above the control levels in response to the B-cell lines, Daudi, and A20/2J, but not to the B-cell line 439.4.2. After fixation with glutaraldehyde, Daudi and A20/2J continued to stimulate this γδ T-cell line. 2CBET-3 cells also responded to the keratinocyte line PAM212, but not to another, XB-2. When lipopolysaccharides (LPS) from *Escherichia coli* or *S. typhimurium* were added to 2CBET-3 cells in the presence of A20/2J cells, 2CBET-3 cells responded with increased cytokine production compared with the cytokine production in the presence of A20/2J cells alone. 2CBET-3 cells by themselves did not respond to LPS alone or to supernatants from A20/2J cells incubated with LPS. Unlike 2CBET-3, the epidermal T-cell hybridoma 70BET-49, expressing a Vγ5/Vδ1-TCR identical to that of 2CBET-3, did not respond to A20/2J cells in the presence or absence of LPS, suggesting a requirement for molecules other than the TCR for Vγ5/Vδ1-TCR$^+$ T-cell stimulation by the B-cell lines and by LPS. This unique reactivity of γδ-TCR$^+$ cells is different from that of αβ-TCR$^+$ cells and may reflect a functional specialization of γδ-TCR$^+$ cells in the response to bacterial infections. *Key words: T lymphocyte/B lymphocyte. J Invest Dermatol 105:58S–61S, 1995*

Most murine epidermal T cells, having a dendritic morphology *in vivo*, express a uniform T-cell receptor (TCR) composed of a Vγ5 and a Vδ1 gene product [1]. A minor population of epidermal T cells expresses Vγ1/Vδ6.3 TCRs and responds to the heat shock protein HSP-60 [2]. However, Vγ5/Vδ1-TCR$^+$ epidermal T cells do not respond to HSP-60, and the ligands that they recognize have remained elusive. Investigations thus far have shown that Vγ5/Vδ1-TCR$^+$ epidermal T cells respond to fresh keratinocytes and to the keratinocyte cell line PAM212 [3], as well as to heat-shocked keratinocytes,†† but not to spleen cells and fibroblasts [3]. These data suggested that Vγ5/Vδ1-TCR$^+$ epidermal T-cell responsiveness is restricted to keratinocytes. However, an observed response of epidermal T-cell clones to the B-cell lymphoma cell line A20/2J (R. E. Tigelaar, unpublished data) led us to investigate whether epidermal T cells can respond to other cell types.

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Because Vγ5/Vδ1-TCR$^+$ epidermal T cells are restricted to the epidermis and are therefore likely to serve as a first line of defense, they may respond to pathogens or to the products of pathogens, such as lipopolysaccharides (LPS), a constituent of gram-negative bacteria. Little information exists as to the LPS responsiveness of T cells. A characteristic that distinguishes B cells from T cells is that at least 30% of B cells are stimulated by LPS, independent of T-cell help, whereas T cells as a rule do not respond to LPS (reviewed in [4]). However, a few studies have emerged suggesting that an LPS-reactive population of T cells exists. A clonal T-cell line, CT 6, proliferates in response to LPS without requiring the presence of other cells [5]. These investigators also found that a small population (approximately 3%) of murine splenic T cells proliferates in response to LPS. Similarly, gram-negative bacteria-reactive T cells appear to respond to the LPS portion of the bacteria [6]. None of these studies addressed whether the LPS-reactive T cells express αβ-TCRs or γδ-TCRs. We show herein that Vγ5/Vδ1-TCR$^+$ epidermal T cells respond to LPS in the presence of A20/2J cells.

MATERIALS AND METHODS

Monoclonal Antibodies The following antibodies were used: 403A.10 (anti-pan γδ-TCR) [7], 17D1 (anti-Vγ5/Vδ1),†† 536 (anti-Vγ5) [8], and 145-2C11 (anti-CD3) [9].

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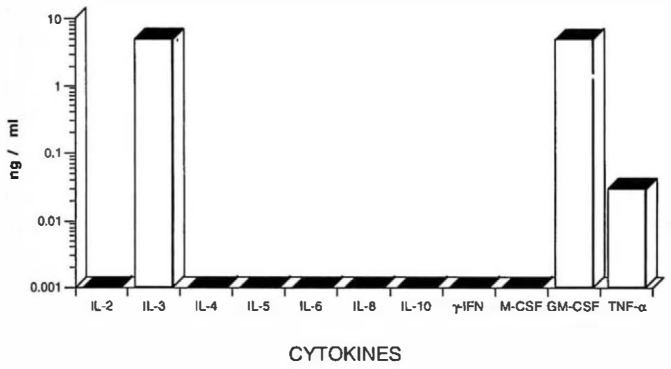

Figure 1. Cytokine profile of the Vγ5/Vδ1-TCR$^+$ epidermal T-cell clonal line 2CBET-3. Cytokines were measured by cytokine-specific enzyme-linked immunosorbent assays, in which concentrations were determined in ng/ml. γ-IFN, interferon-γ; TNF-α, tumor necrosis factor-α.

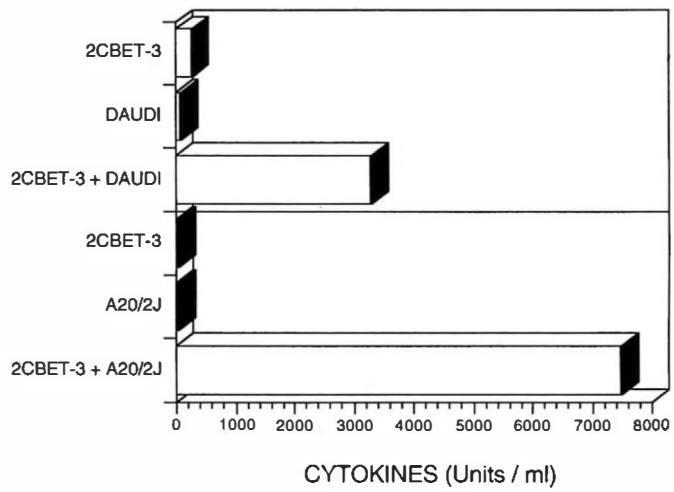

Figure 2. Murine and human B-cell lines stimulate 2CBET-3. 2CBET-3 cells (3×10^5/well) were mixed with Daudi or A20/2J cells (1×10^5/well) in microtiter wells. The culture medium was removed and assessed for the production of IL-3 and GM-CSF using the DA-1 bioassay, as described in *Materials and Methods*. The data represent one of five replicate experiments.

T-Cell Clonal Line Production and Analysis Epidermal cells were prepared from C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME), as described previously [10]. Briefly, whole trunk or ear skin was trypsinized overnight at 4°C. Epidermal cells were gently separated from the dermis and trypsinized at 37°C to produce single-cell suspensions. After nylon wool passage to remove non-T cells, the cells were cultured in 24-well plates, coated with pan–anti-γδ TCR [7] together with 10 U/ml recombinant IL-2 (R and D Systems, Minneapolis, MN), for 72 h. Clonal T-cell lines were produced by limiting dilution. The epidermal T-cell clonal lines were stained with the above monoclonal antibodies, followed by single-color cytofluorometric analysis to confirm that they expressed the Vγ5/Vδ1-TCR.

Stimulator Cell Lines 2CBET-3 cells were incubated with a variety of murine cell lines, including fibroblast cell lines NIH3T3 and L929, trophoblast cell line TR-B, melanoma cell line B16FO, embryonic carcinomas PCC3 and PCC4, B-cell lymphomas 439.4.2 and A20/2J, keratinocyte cell lines XB-2 and PAM212, mastocytoma line P815, and the human B-lymphoma cell line Daudi.

Cell Fixation Stimulator cells (5×10^6 total), suspended in balanced salt solution, were fixed with glutaraldehyde (0.05% v/v) for 30 seconds and mixed 1:1 with 0.2 M lysine, pH 7.4, in balanced salt solution to stop the reaction. Cells were washed further with balanced salt solution before incubation with responder T cells.

Analysis of Epidermal T-Cell Cytokine Production Cytokines were identified by specific enzyme-linked immunosorbent assay techniques at the DNAX Research Institute, as described previously [11].

Assessment of Epidermal T-Cell Reactivity Responses to the various cell lines or to LPS were detected by cytokine production by 2CBET-3. Because 2CBET-3 makes interleukin-3 (IL-3) and granulocyte–macrophage colony-stimulating factor (GM-CSF) **(Fig 1)**, culture supernatants from experiments involving 2CBET-3 were tested on the IL-3/GM-CSF–dependent DA-1 cell line, as described previously [12]. One unit of these cytokines was defined as equivalent to 0.001 U of recombinant GM-CSF (Genzyme). Recombinant GM-CSF was used as a standard in all assays. Units of IL-2 in the culture supernatants of experiments involving the epidermal T-cell hybridoma 70BET-49 [2] were measured using the cytokine-dependent HT-2 cell line, as described previously [12].

LPS LPS from *Escherichia coli* serotype 055:B5 and *S. typhimurium* SL1181 Re mutant (Sigma Chemical Co., St. Louis, MO) were added to A20/2J cells and to 2CBET-3 at the start of the culture.

RESULTS AND DISCUSSION

The Vγ5/Vδ1-TCR$^+$ epidermal T-cell clone 2CBET-3 was tested to determine which cytokines are produced after activation of these T cells. When stimulated by TCR cross-linking, 2CBET-3 produced IL-3, GM-CSF, and tumor necrosis factor-α **(Fig 1)**. No detectable production of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, or interferon-γ was found. Therefore, we used an IL-3– and GM-CSF–dependent cell line, DA-1, to assess stimulation of 2CBET-3 in further experiments.

2CBET-3 was then tested for responses to a variety of cell lines, as described below. Of all the cells tested, 2CBET-3 responded strongly to only two cell lines: the murine B-cell lymphoma cell line A20/2J and the human B-cell lymphoma Daudi **(Fig 2)**. A different Vγ5/Vδ1-TCR$^+$ epidermal T-cell clone, 2CBET-1, responded similarly to A20/2J and Daudi cells (data not shown). Fixation of A20/2J and Daudi cells did not interfere with their ability to stimulate 2CBET-3 **(Fig 3)**. Thus, the epidermal T-cell response was not due to cytokines made by the stimulator cells. 2CBET-3 cells were not or were only weakly stimulated by other cell lines, including the murine B-cell line 439.2.4, the fibroblast cell lines 3T3 and L929, the trophoblast cell line TR-B, the melanoma cell line B16FO, embryonic carcinomas PCC3 and PCC4, and the mastocytoma line P815 **(Fig 4)**.

2CBET-3 also responded, albeit to a lesser degree, to the murine

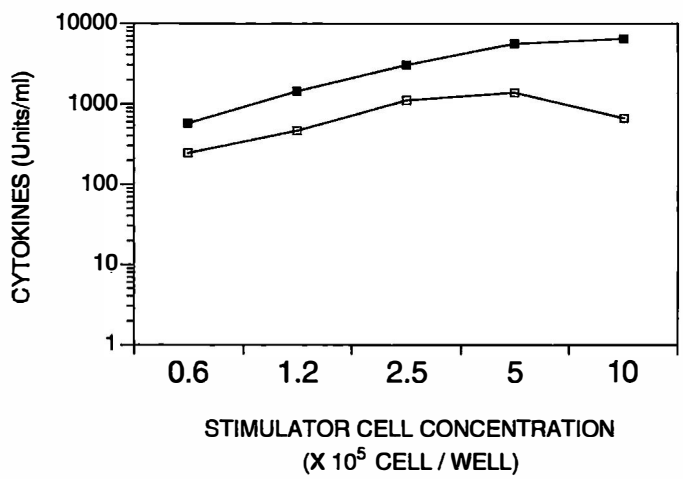

Figure 3. 2CBET-3 responds to fixed B-cell lines. 2CBET-3 cells (3×10^5/well) were mixed with A20/2J (*closed squares*) and Daudi (*open squares*) cells that were fixed with glutaraldehyde, as described in *Materials and Methods*. The culture medium was removed and assessed for the production of IL-3 and GM-CSF using the DA-1 bioassay. Greater concentrations of the fixed B-cell lines were required for maximal responses to occur, compared with assays using live cells. The data represent one of three replicate experiments.

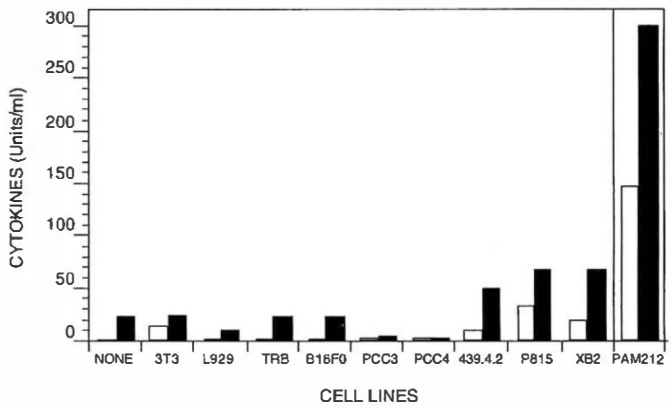

Figure 4. Various cell lines do not stimulate 2CBET-3. 2CBET-3 cells (3×10^5/well) were mixed with a variety of murine cell lines (1×10^5/well) in microtiter wells, including fibroblast cell lines NIH3T3 and L929, trophoblast cell line TR-B, melanoma cell line B16F0, embryonic carcinomas PCC3 and PCC4, B-cell lymphoma 439.4.2, mastocytoma line P815, and the keratinocyte cell lines XB-2 and PAM212. The culture medium was removed from these mixtures and assessed for the production of IL-3 and GM-CSF using the DA-1 bioassay. *Open bars*, cytokine production by the cell lines alone; *closed bars*, cytokine production by 2CBET-3 cells mixed with the cell lines. The data represent one of three replicate experiments.

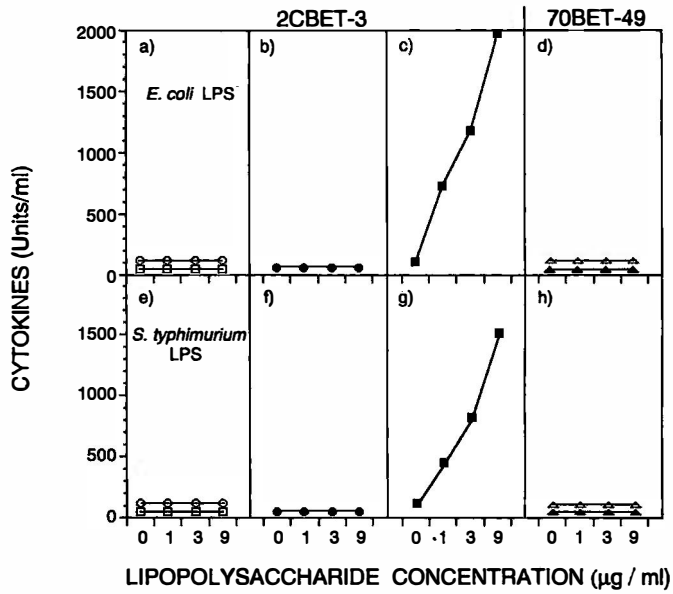

Figure 5. 2CBET-3 is stimulated by bacterial LPS in the presence of A20/2J cells. Various concentrations of LPS from either *E. coli* (*a,b,c,d*) or *S. typhimurium* (*e,f,g,h*) were added at the start of the culture to 2CBET-3 cells (3×10^5/well) mixed with A20/2J cells (1×10^5/well), as before. The data reflect LPS responses of 2CBET-3 alone (*a,e; open squares*) and A20/2J cells alone (*a,e; open circles*), of 2CBET-3 cells incubated with culture supernatants from A20/2J cells that had been incubated with LPS for 24 h (*b,f; closed circles*), of 2CBET-3 together with A20/2J cells (*c,g; closed squares*), and of the Vγ5/Vδ1-TCR$^+$ hybridoma 70BET-49 (*d,h*) together with (*closed triangles*) and without (*open triangles*) A20/2J cells. The culture medium was removed from these mixtures and assessed for the production of IL-3 and GM-CSF using the DA-1 bioassay. Cytokine values shown near 100 U/ml reflect less than 30 U/ml. These data represent one of three replicate experiments.

keratinocyte line PAM212, but showed little reactivity with the murine keratinocyte line XB-2 **(Fig 4)**. The reactivity to PAM212 is consistent with the data of Havran *et al* [3], in which the epidermal T-cell line 7-17 was found to respond strongly to PAM212. Differences appeared to exist between various Vγ5/Vδ1-TCR$^+$ epidermal T-cell clones in their reactivity to A20/2J and PAM212 cells, with some clones responding preferentially to the B-cell lines and others to keratinocytes (R. E. Tigelaar, unpublished data). However, 2CBET-3 responses to A20/2J cells varied considerably in strength, as can be seen by comparing the responses shown in **Fig 2** and **Fig 5**. The reason for the variability of responses to A20/2J is not clear, but could be related to cell densities at which the 2CBET-3 (data not shown) and other Vγ5/Vδ1-TCR$^+$ epidermal T-cell clones (R. E. Tigelaar, unpublished data) were maintained before their incubation with A20/2J cells, such that cell cultures maintained at higher densities displayed better responses. Because all of these responding cells expressed the same TCR and similar levels of TCR expression (data not shown), the variable reactivity may reflect differences in expression of accessory or adhesion molecules or alternative ligands on the Vγ5/Vδ1-TCR$^+$ epidermal T-cell clones. In contrast to the responses to A20/2J cells, the responses of 2CBET-3 to PAM212 cells have been consistently small.

Because murine Vγ5/Vδ1-TCR$^+$ epidermal T cells respond to both a mouse and a human B-cell line, they may recognize a highly conserved ligand. Similarly, Vγ1-TCR$^+$ cells respond to both murine and human trophoblast cells and also may recognize a highly conserved ligand [13]. The ligand recognized by Vγ5/Vδ1-TCR$^+$ epidermal T cells may not be unique to a particular type of cell, as the same epidermal T cells respond to keratinocytes. This view contrasts with that of Havran *et al* [3], who proposed that Vγ5/Vδ1-TCR$^+$ epidermal T cells recognize a ligand specific to keratinocytes. Alternatively, epidermal T cells may recognize not a single ligand but rather a set of related ligands expressed on different cells. It is interesting that Vγ5/Vδ1-TCR$^+$ epidermal T cells do not respond to all keratinocyte and B-cell lines. This finding is reminiscent of the polyclonal responses of human Vγ9/Vδ2-TCR$^+$ T-cell clones, which respond only to certain B-cell lines, particularly the Burkitt's lymphoma line Daudi [14]. Such a variable capability of B-cell lines to stimulate γδ T cells could reflect variable ligand expression or the expression of accessory molecules such as the integrins or the costimulatory B7 molecules by the B-cell lines (reviewed in [15,16]).

Because of the likely exposure of epidermal T cells to pathogens from the external environment, 2CBET-3 was assessed for its ability to respond to bacterial LPS. 2CBET-3 cells responded to LPS in the presence of A20/2J cells, but not in their absence **(Fig 5)**. They responded similarly to LPS from two different bacterial species, *E. coli* and *S. typhimurium*. By themselves, the A20/2J cells incubated with LPS did not produce cytokines stimulatory for DA-1 cells **(Fig 5)**. Because the 2CBET-3 response could be due to cytokines produced by LPS-activated A20/2J cells, we tested the ability of culture supernatants from A20/2J cells incubated with LPS for 24 h to stimulate the Vγ5/Vδ1-TCR$^+$ cells in the absence of A20/2J cells. No response was detected **(Fig 5)**. Moreover, the necessity for A20/2J cells for the 2CBET-3 response to LPS is underscored by the finding that Vγ5/Vδ1-TCR$^+$ epidermal T cells do not respond to LPS adherent to tissue culture plastic (data not shown). We also note that although the 2CBET-3 cells responded weakly to keratinocyte lines, evaluation of a response to LPS in the presence of these cell lines is clouded by the ability of the keratinocyte lines to produce IL-3 in response to LPS (personal observation).

In contrast to the LPS response by the Vγ5/Vδ1-TCR$^+$ clone 2CBET-3, the Vγ5/Vδ1-TCR$^+$ hybridoma 70BET-49 did not respond to LPS under similar conditions, even up to concentrations of 100 μg/ml (data not shown), or to A20/2J cells alone **(Fig 5)**. Nevertheless, this cell does produce cytokines in response to TCR cross-linking with anti-TCR antibodies, suggesting that 70BET-49 lacks qualities other than Vγ5/Vδ1-TCR expression that are essential for the responses to LPS and to the B-cell lines. However, the hybridoma fusion partner, BW5147, is known to suppress the T-cell surface molecule CD8 [17], and therefore it also might

suppress other accessory molecules required for Vγ5/Vδ1-TCR$^+$ cell responses.

The observation that Vγ5/Vδ1-TCR$^+$ epidermal T cells are able to respond to bacterial LPS in the presence of A20/2J cells distinguishes Vγ5/Vδ1-TCR$^+$ epidermal T cells from the majority of T lymphocytes, which do not respond to LPS [5]. However, LPS responsiveness does not appear to be unique to Vγ5/Vδ1-TCR$^+$ epidermal T cells, as murine γδ-TCR$^+$ cell lines expressing other TCRs also were found to be reactive with LPS in the presence of accessory cells (M. Tsuji and F. Zavala, data not shown). Thus, the mechanism of LPS stimulation is not likely to involve LPS recognition by the TCR. Nevertheless, the sensitivity of γδ-TCR$^+$ cells to LPS stimulation represents an important difference between these cells and αβ-TCR$^+$ cells, and likely reflects different roles of the two T-cell subsets *in vivo*. Possibly, stimulation of γδ-TCR$^+$ cells by LPS (and perhaps by other bacterial products) could initiate a cascade of events that affect the host and the pathogen in a scenario comparable to superantigen-induced polyclonal αβ-TCR$^+$ cell responses.

Several possibilities exist for the mechanism of stimulation of epidermal T cells by LPS in association with A20/2J cells. 2CBET-3 cells either respond to LPS "presented" in some form by A20/2J cells or respond to another ligand on A20/2J cells whose stimulatory activity (e.g., conformation or levels of expression) is induced or modified by LPS. Although the LPS molecule is not polyvalent *per se*, it tends to form complexes in aqueous solution, which could nonspecifically "glue" the 2CBET-3 and A20/2J cells together. In contrast to nonspecific interactions with LPS, A20/2J cells may express LPS bound to a receptor, such as CD14, CD11/CD18, or the 80-kDa LPS-binding protein, and thus the Vγ5/Vδ1-TCR$^+$ epidermal T cells could recognize receptor-bound LPS *via* a member of the CD11/CD18 LPS receptor family (reviewed in [18]). In either case, LPS may serve only to enhance other cell-surface interactions that lead to epidermal T-cell activation by the A20/2J cells. Therefore, one might expect that other cells expressing LPS receptors similar to those on A20/2J cells, e.g., macrophages, would be able to function in place of A20/2J cells. Alternatively, LPS may act on either the epidermal T cells or the A20/2J cells to induce or increase the expression of accessory molecules important to the response, such as adhesion molecules. Also, considering the suggestions of heat shock proteins as possible endogenous ligands for Vγ5/Vδ1-TCR$^+$ epidermal T cells [1], LPS has been shown recently to induce expression of HSP-70 in macrophages after LPS administration *in vivo* [19]. Perhaps LPS induces expression of HSP-70 or other heat shock proteins on A20/2J cells, to which the 2CBET-3 cells respond.

The ligand on A20/2J cells that stimulates Vγ5/Vδ1-TCR$^+$ epidermal T cells remains unknown at the present time. However, the response to LPS in association with A20/2J cells may offer clues as to the nature of interactions not only between γδ T cells and bacterial pathogens, but also between γδ T cells and other cells of the host. That Vγ5/Vδ1-TCR$^+$ epidermal T cells are activated by some B-cell lines *in vitro* may reflect the ability of these epidermal T cells to interact with B lymphocytes *in vivo*, and such interactions, which have yet to be described, may be important during various infections or other pathologic conditions.

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REFERENCES

1. Asarnow DM, Kuziel WA, Bonyhadi M, Tigelaar RE, Tucker PW, Allison JP: Limited diversity of γδ antigen receptor genes of Thy-1$^+$ dendritic epidermal cells. *Cell* 55:837–847, 1988
2. Reardon CL, Vollmer M, Cranfill R, O'Brien RL, Born WK: Response of a murine epidermal Vγ5/Vδ6-TCR$^+$ hybridoma to heat shock protein, HSP-60. *J Invest Dermatol* 103:544–546, 1994
3. Havran WL, Chien Y-H, Allison JP: Recognition of self antigens by skin-derived T cells with invariant γδ antigen receptors. *Science* 252:1430–1432, 1991
4. Morrison D, Ryan JL: Bacterial endotoxins and host immune response. *Adv Immunol* 28:294–431, 1979
5. Vogel SN, Hilfiker ML, Caulfield MJ: Endotoxin-induced T lymphocyte proliferation. *J Immunol* 130:1774–1779, 1983
6. Jirillo E, De Simone C, Covelli V, Kiyono H, McGhee JR, Antonaci S: LPS-mediated triggering of T lymphocytes in immune response against gram-negative bacteria. *Adv Exp Med Biol* 256:417–425, 1990
7. Itohara S, Nakanishi N, Kanagawa O, Kubo R, Tonegawa S: Monoclonal antibodies specific to native murine T-cell receptor γδ: Analysis of γδ T cells during thymic ontogeny and in peripheral lymphoid organs. *Proc Natl Acad Sci USA* 86:5094–5098, 1989
8. Havran WL, Grell S, Duwe G, Kimura J, Wilson A, Kruisbeek AM, O'Brien RL, Born W, Tigelaar RE, Allison JP: Limited diversity of TCR γ-chain expression of murine Thy-1+ dendritic epidermal cells revealed by Vγ3-specific monoclonal antibody. *Proc Natl Acad Sci USA* 86:4185–4189, 1989
9. Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA: Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc Natl Acad Sci USA* 84:1374–1378, 1987
10. Nixon-Fulton JL, Bergstresser PR, Tigelaar RE: Thy-1$^+$ epidermal cells proliferate in response to concanavalin A and interleukin 2. *J Immunol* 136:2776–2786, 1986
11. Abrams JS: Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anticytokine antibodies. In: Colligan JE, Kruisbeek AM, Margules DK, Shevach EM (eds.). *Current Protocols in Immunology*. John Wiley and Sons, New York (in press)
12. O'Brien RL, Happ MP, Dallas A, Palmer E, Kubo R, Born WK: Stimulation of a major subset of lymphocytes expressing T cell receptor γδ by an antigen derived from Mycobacterium tuberculosis. *Cell* 57:667–674, 1989
13. Heyborne K, Fu X-Y, Nelson A, Farr A, O'Brien R, Born W: Recognition of trophoblasts by γδ T cells. *J Immunol* 153:2918–2926, 1994
14. Fisch P, Malkovsky M, Kovats S, Sturm E, Braakman E, Klein BS, Voss SD, Morrissey LW, DeMars R, Welch WJ, Bolhuis RLH, Sondel PM: Recognition by human Vγ9/Vδ2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. *Science* 250:1269–1273, 1990
15. Dustin M, Springer TA: Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. *Annu Rev Immunol* 9:27–66, 1991
16. June CH, Bluestone JA, Nadler LM, Thompson CB: The B7 and CD28 receptor families. *Immunol Today* 15:321–331, 1994
17. Burgert HG, White J, Weltzien HU, Marrack P, Kappler JW: Reactivity of VB17A+ CD8+ T cell hybrids: analysis using a new CD8+ T cell fusion partner. *J Exp Med* 170:1887–1904, 1989
18. Ulevitch RJ: Recognition of bacterial endotoxins by receptor-dependent mechanisms. *Adv Immunol* 53:267–289, 1993
19. Zhang Y-H, Takahashi K, Jiang G-Z, Zhang X-M, Kawai M, Fukada M, Yokochi T: In vivo production of heat shock protein in mouse peritoneal macrophages by administration of lipopolysaccharide. *Infect Immun* 62:4140–4144, 1994